Article

Biosecurity practices and causes of enteritis on Ontario meat rabbit farms

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Abstract — Infectious enterocolitis is a significant cause of mortality in meat rabbits. Disease risk is enhanced by intensive rearing practices and poor on-farm biosecurity. This investigation was undertaken in farmed meat rabbits during an Ontario-wide outbreak of enteritis with high mortality to determine the prevalence of causative agents. A survey evaluating on-farm biosecurity practices was also conducted to identify potential means of pathogen contamination and zoonotic risks. Gross and microscopic pathology evaluations combined with microbiologic testing were conducted on 95 rabbits over spring and winter months. *Escherichia coli* and *Clostridium spiroforme* were most commonly associated with enteritis in rabbits regardless of age or season and lesions were significantly more severe in mature does ($P < 0.0001$). The survey results demonstrated a lack of consistent on-farm biosecurity practices. The infectious nature of enteric disease of rabbits combined with poor biosecurity practices may contribute to disease transmission within and between farms.

Résumé — Pratiques de biosécurité et causes d'entérite dans des fermes d'élevage de lapins de l'Ontario. L'entérocolite infectieuse est une cause importante de mortalité chez les lapins d'élevage. Le risque de maladie est accru par des pratiques d'élevage intensives et une mauvaise biosécurité à la ferme. Cette enquête a été entreprise chez des lapins d'élevage durant une éclosion d'entérite à l'échelle de l'Ontario qui présentait un taux de mortalité élevé afin de déterminer la prévalence des agents étiologiques. On a aussi réalisé un sondage évaluant les pratiques de biosécurité à la ferme afin d'identifier les modes potentiels de contamination des agents pathogènes et les risques zoonotiques. Des évaluations pathologiques macroscopiques et microscopiques combinées à des tests microbiologiques ont été réalisés sur 95 lapins au cours des mois d'été et d'hiver. *Escherichia coli* et *Clostridium spiroforme* étaient le plus communément associés à l'entérite chez les lapins sans égard à l'âge ou à la saison et les lésions étaient significativement plus graves chez les lapines adultes ($P < 0{,}0001$). Les résultats du sondage ont démontré l'absence de pratiques de biosécurité uniformes à la ferme. La nature infectieuse de la maladie entérique des lapins et de mauvaises pratiques de biosécurité peuvent contribuer à la transmission de la maladie dans les fermes et entre ces dernières.

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Introduction

The meat rabbit industry is a small but important food animal commodity group in Canada, providing a relatively inexpensive and nutritious protein source, and an alternate protein source for companion animal feed. Ontario processed over 65% of the 664 711 rabbits slaughtered for food in Canada in 2015 (1). Ontario rabbit producers have struggled to meet consumer demands and importation has been necessary (1). Many Ontario rabbit farms are small, family-run operations in which annual pre-market mortality risks have remained steady at approximately 20% to 25%, exceeding accepted levels for other food animal groups (2). This is consistent with levels of mortality seen in meat rabbitries worldwide and is largely due to infectious enteric and respiratory diseases. There has been minimal veterinary support for this sector in Canada and few guidelines to assist with improving on-farm management and husbandry practices.

Enteritis, more appropriately termed rabbit enteritis complex (REC) since the pathogenesis is multifactorial, remains

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a significant issue plaguing the meat rabbit sector. The most common causes of REC in young growing rabbits include *E. coli, Clostridium* spp., *Lawsonia intracellularis,* and coccidia; co-infection with 2 or more pathogens is common (3–6). The specific prevalence of these agents is unknown in Canada. Viruses (e.g., rotavirus, coronavirus, astrovirus) may also be co-factors in disease (4,5). These organisms are all transmitted by the orofecal route, but factors such as diet, temperature extremes, maternal antibody levels, and genetic background can influence susceptibility (3,7,8). Several REC-inducing agents, such as *C. difficile* and certain strains of *E. coli,* also cause gastrointestinal and other illnesses in humans, suggesting that meat rabbits may be a potential reservoir for zoonotic pathogens. A recent study suggested that 25% of rabbits within a US meat rabbitry were culture- and polymerase chain reaction (PCR)-positive for enterohemorrhagic *E. coli,* an agent associated with hemorrhagic colitis and hemolytic uremic syndrome in humans (9). Meat rabbits are also sold as pets and for research use, thus broadening potential human exposures.

The high incidence of infectious enteric disease within meat rabbitries raises questions with respect to on-farm disease prevention and management practices. Factors that may contribute to the persistence of infectious diseases include the intensive nature of rabbit farming, a lack of efficacious vaccines for common diseases, and a lack of efficacious antimicrobial agents licensed for use in meat rabbits. Currently, there are only 4 therapeutics licensed for use in meat rabbits in Canada, 3 for coccidiosis and 1 for respiratory disease, resulting in significant off-label antimicrobial use. Because rabbits are hindgut fermenters, antimicrobials given can result in alterations in gastrointestinal microbiota, inducing dysbiosis and predisposing animals to enteric disease. It is well-known that inappropriate use of antimicrobial agents can lead to antimicrobial resistance (AMR) (10,11). Whether antimicrobial resistant bacteria are present in meat rabbits has not been evaluated in Canada, but AMR has been demonstrated for *E. coli, Salmonella* spp., and *Staphylococcus aureus* in meat rabbits in other countries, including Italy, Portugal, Belgium, France, the UK, the Netherlands, and Spain (12–14). Diets low in fiber and high in digestible carbohydrates, compared to those fed to companion rabbits, are routinely fed to meat rabbits to increase body weight and growth (15); however, such practices can result in an altered gut environment, allowing pathogenic bacteria to proliferate (7,8,16). In addition, high density settings enable disease agents, such as *Pasteurella multocida, E. coli,* and *Clostridium* spp. to be readily transmitted among animals (17).

Specific biosecurity measures can significantly decrease infectious diseases, and aid in disease control. Biosecurity can be loosely defined as a "series of management practices designed to prevent, minimize, and control the introduction, spread, and release of... pests" (18). After introduction of new animals to a facility, human-assisted movement of pathogens is thought to be a major cause of biosecurity problems on farms (19). The steps involved in implementing biosecurity on-farm can be categorized into 3 major areas: i) access management, which includes managing farm visitors and their movement between areas, as well as access to other animal species; ii) animal health management, which refers to monitoring for and treatment of disease, establishing protocols for quarantine and animal movement, and plans for managing disease situations; and iii) operation management, which includes disposal practices for manure and deadstock, measures taken to keep facilities clean and in good repair, pest control measures, and food and water processing and storage (18). Introduction of these biosecurity measures is expected to decrease introduction and spread of diseases on-farm. Because of this, many Canadian food animal production groups have introduced farm-level biosecurity standards for their respective industries. Unfortunately, no such standards exist for the Canadian meat rabbit industry.

An opportunity to characterize causes of infectious enteritis in meat rabbits and associated producer biosecurity measures occurred in Ontario during a province-wide outbreak of enteritis, in which mortality risks of growing animals exceeded 40% on some farms. Affected rabbits had persistent, severe, watery diarrhea with dehydration and wasting. The acute and widespread onset of the enteritis suggested that 1 or more infectious agents were likely, but further diagnostic tests and epidemiologic measures were needed to identify the underlying causes. Thus, the goals of this study were to: i) identify the prevalence of specific infectious causes of enteritis in Ontario meat rabbits, and ii) characterize on-farm biosecurity practices.

Materials and methods

Part A: Disease surveillance — Causes of infectious enteritis in Ontario commercial meat rabbits

Animals. A total of 95 meat rabbits were submitted for routine postmortem examination to the Animal Health Laboratory (AHL), University of Guelph between May 2007 and February 2008, during an industry-wide enteritis outbreak in Ontario. Rabbit producers were contacted by University of Guelph researchers through the Ontario Commercial Rabbit Growers Association (OCRGA) to submit live rabbits in groups of 3 from 2 age groups (growing market rabbits or fryers and mature breeding does) that were exhibiting signs of acute diarrhea. Evaluations were grouped based on the time of year in which submissions were received.

Postmortem procedures. Following euthanasia, cadavers were weighed and gross examinations were conducted. Tissue samples were collected from the liver, jejunum, ileum, cecum, and colon into 10% neutral buffered formalin and were routinely trimmed, processed, sectioned, and stained with hematoxylin and eosin for microscopic evaluation. Fresh cecal contents were submitted for aerobic and anaerobic culture and antimicrobial susceptibility testing for bacteria including *E. coli, Salmonella* spp., and *Clostridium* spp. The microbiology, excluding culture for *C. difficile,* was conducted by the AHL according to their standard procedures, which included the use of enrichment broth for isolation of *Salmonella* spp. The presence of *Clostridium spiroforme* was identified by Gram stain of fecal material. Polymerase chain reaction was used to confirm the presence of *C. spiroforme, C. perfringens,* and *L. intracellularis.* Any positive *Salmonella* spp. isolates were forwarded to the Laboratory for Foodborne Zoonosis, Public Health Agency of Canada, Guelph, Ontario

Table 1. Scoring system for intestinal histopathology

Inflammation		
	0	None
	1	Minimal — small/large intestine: Occasional lymphocyte or eosinophil within the lamina propria
	2	Mild — multifocal infiltrates within mucosa, predominantly lymphocytic or eosinophilic (< 25% affected), minimal edema within the lamina propria
	3	Moderate — increased inflammation (25% to 65%) with edema in mucosa (+ neutrophils)
	4	Marked — extensive inflammation within mucosa and submucosa with abundant edema in both
Mucosal necrosis		
	0	None
	1	Minimal — occasional epithelial tufting, rare single cell necrosis
	2	Mild — multifocal tufting and/or single cell necrosis (< 25% affected), mild villus fusion, and/or atrophy
	3	Moderate — single cell necrosis, atrophy/fusion (25% to 65% affected), upper third of villus tips, extensive epithelial loss with attenuation of epithelium, focal hemorrhage, loss of goblet cells in upper third mucosa (colon)
	4	Marked — > 65% affected, > 50% villus height reduction
Gland/crypt morphology (chronicity)		
	0	None — straight, tightly packed glands/crypts with occasional mitotic figure
	1	Minimal — mild increase in mitotic figures in crypts/glands
	2	Mild — focal mucosal hyperplasia (< 25% affected), crypt dilation, loss of goblet cells
	3	Moderate — multifocal mucosal hyperplasia (coiling glands), loss of goblet cells
	4	Marked — as above + crypt abscesses
Erosion		
	1	present
Bacteria — scored as present/not		

for serotyping. *Escherichia coli* isolates were tested for presence of genes for intimin *(eae)* and Shiga toxin (*stx1* and *stx2*). Isolates positive for the *eae* gene only were classified as enteropathogenic *E. coli* (EPEC), whereas isolates positive for any Shiga toxin gene with or without *eae* gene were characterized as verotoxigenic *E. coli* (VTEC). To identify *C. difficile,* samples of cecal contents were processed according to Fedorko and Williams (20).

Histopathology evaluations. Sections of jejunum, ileum, cecum, and colon were scored for level of inflammation, degree of mucosal necrosis, gland/crypt morphology, and presence of mucosal erosion by 2 pathologists (MB, PVT) who were blind to the other data (Table 1). A total histopathology score for each intestinal section was calculated by tallying the scores for each criterion with a maximum score of 13. Qualitative histologic changes in liver sections were recorded.

Part B: Industry biosecurity survey

A 60-question survey concerning on-farm animal husbandry and biosecurity practices was developed and mailed to 50 commercial rabbit producers through OCRGA. The survey was approved by the University of Guelph Research Ethics Board (07AU033) and participation was voluntary. In addition to basic farm information and background, questions in the survey focused on the 3 main areas of biosecurity: i) access management, ii) animal health management, and iii) operations management.

Statistical analyses. Statistical analyses were conducted using SAS version 9.2 (StataCorp, Cary, North Carolina, USA). Histologic scores were compared using a general linear mixed model (Proc Mixed) with 3 fixed effects (season, age, and sample site) and least squares means analysis was used for comparison. Analysis of variance (ANOVA) assumptions were examined using residual analyses, including testing residuals for normality, and plotting residual against predicted values and explanatory variables. For the disease surveillance data, odds ratios (OR) and 95% confidence intervals (CI) were calculated for each bacterium, comparing the prevalence of the bacterium in the spring *versus* winter cohorts, and in fryers *versus* does. In cases in which the odds ratio was equal to zero or infinity, a median unbiased estimate was substituted (21). Results were considered significant when $P < 0.05$.

For analysis, descriptive questions from the survey were re-formatted into yes/no questions and responses were coded accordingly. Farm size was divided into "small" (≤ 200 breeding does) and "large" (> 200 breeding does). Results were formatted into 2 × 2 tables and OR with 95% CI were calculated.

Results

Part A: Disease surveillance — Causes of infectious enteritis in Ontario commercial meat rabbits

Pathologic evaluations. Forty animals, most with diarrhea, were submitted between May and June of 2007. Animals included 15 does and 21 fryers from 6 commercial farms and 4 healthy rabbits (2 does and 2 fryers) submitted from a 7th farm. The 4 healthy rabbits were excluded from final analyses and were used as histology controls only. Grossly, affected rabbits were moderately dehydrated with marked perianal fecal staining (Figure 1A). The small and large intestines often contained varying quantities of clear to green liquid (Figure 1B). Occasionally, formed fecal pellets were present within the descending colon and rectum. Additional non-enteric findings in these animals included hepatic abscesses or abscesses scattered within the abdomen, and purulent otitis media. In most rabbits, microscopic gastrointestinal changes consisted of patchy to segmental to generalized mucosal inflammation and edema with erosive to ulcerative typhlitis and colitis (Figure 1C). A range of infectious agents was detected microscopically and subsequently confirmed by ancillary testing including *E. coli* (EPEC and non-EPEC), *L. intracellularis, C. spiroforme,* and sexual and asexual coccidial forms (Figure 1D).

Between late November 2007 and February 2008, 37 fryers and 18 does were submitted for evaluation. Of these 55 animals, 6 fryers and 6 does were excluded from the final analysis as there was no evidence of diarrhea on gross or microscopic examination. All animals that were included in the final analysis were moribund on presentation. The gross and microscopic appearance of tissues from these animals was consistent with the spring cohort with the addition of erosion or ulceration of the plantar aspect of the hocks.

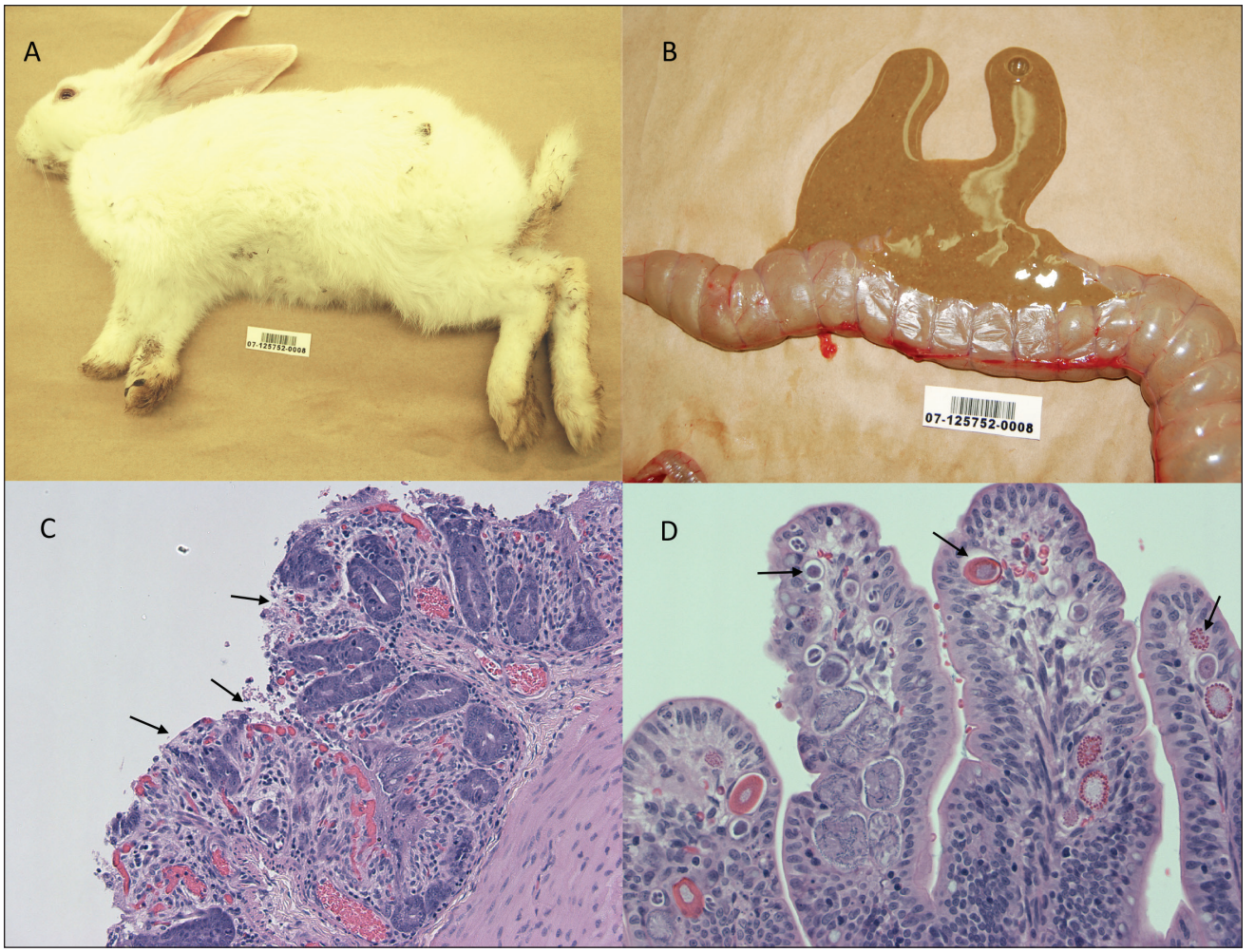

Figure 1. A – common presentation of enteritis ('soiled hocks') in a young growing rabbit (fryer). B – cecum from a fryer with enteritis, depicting typical moderate dilatation and watery content. C – Photomicrograph of cecum from a fryer infected with nonenteropathogenic *E. coli* demonstrating generalized mucosal erosion (arrows) with congestion and mixed leukocytic infiltrates, H&E, 200×. D – photomicrograph of jejunum from a fryer with enteric coccidiosis demonstrating numerous life cycle stages within the lamina propria (arrows), H&E, 400×.

Significant differences were identified in intestinal histopathology scores between the different age groups ($P < 0.01$) and between intestinal sites ($P < 0.01$). Overall, does had higher mean microscopic lesion scores and thus more severe lesions than fryers [mean lesion score for does: 2.75, lower limit (LL) = 2.25, upper limit (UL) = 3.25; mean lesion score for fryers 2.25, LL = 1.75, UL = 2.75]. When comparing mean microscopic lesion scores for each gut section, severity of scores progressed from the ileum being the least affected (average score = 1.75, LL = 1.04, UL = 2.46), to the jejunum (average score = 2.50, LL = 1.79, UL = 3.21), then the colon (average score = 2.75, LL = 2.04, UL = 3.46), and finally the cecum being the most severely affected region (average score = 3.00, LL = 2.29, UL = 3.71) regardless of age or season. No significant differences in lesion severity were identified between winter and spring samples ($P = 0.36$). Significant two-way interactions were identified when age × sample sites were examined ($P < 0.01$), and when season × sample sites were compared ($P = 0.02$). No significant 3-way interaction was identified when season × age × sample site were compared ($P = 0.06$); however, the common comparator of sample sites for both of the 2-way interactions prevented disentanglement for additional analysis and therefore a 3-way simple effects analysis was still required. Least mean squares of histopathology scores are presented in Table 2 and differences among these are depicted in Table 3.

Microbiology findings. Bacteriology results are summarized in Table 4. *Clostridium spiroforme* and *L. intracellularis* infections were identified exclusively in fryers in both spring and winter cohorts, although *C. spiroforme* was found in 1 winter doe sample. While the odds of isolating *E. coli* did not differ significantly between winter and spring nor between fryers and does, the odds of isolating EPEC (*eae*-positive, *stx*-negative *E. coli*) were significantly higher during the winter than in the spring (OR = 10.36; 95% CI = 1.544 to 233.098; $P = 0.0096$), and for fryers than does (OR = 8.703; 95% CI = 1.299 to 195.866; $P = 0.02$). In addition, the odds of identifying *C. spiroforme* in fryers were significantly higher than in does (OR = 7.63; 95% CI = 1.101 to 173.213; $P = 0.039$).

Salmonella Agona was isolated in 1 doe without diarrhea. Two cases of *L. intracellularis* infection were identified during

Table 2. Least squares means of intestinal histopathology scores for commercial meat rabbits with diarrhea when all variables are included in the comparison

Variable A (season)	Variable B (age)	Variable C (sample site)	Least squares mean estimate	Lower limit	Upper limit
Spring	Doe	Cecum	4.78	3.73	5.82
		Colon	3.98	2.92	2.54
		Ileum	3.13	1.91	4.34
		Jejunum	3.38	2.33	4.42
	Fryer	Cecum	6.40	5.44	7.36
		Colon	5.25	4.29	6.21
		Ileum	4.64	3.59	5.69
		Jejunum	5.35	4.39	6.31
Winter	Doe	Cecum	3.88	2.78	4.98
		Colon	2.96	1.86	4.06
		Ileum	2.68	1.53	3.83
		Jejunum	2.56	1.43	3.68
	Fryer	Cecum	5.40	4.58	6.22
		Colon	4.40	3.58	5.22
		Ileum	4.67	3.84	5.51
		Jejunum	6.50	5.68	7.32

routine microscopic examination; these cases, plus an additional 2 cases, were confirmed using PCR. *Clostridium perfringens* was isolated from 1 fryer sample while *C. difficile* was not isolated from any sample.

Part B: Industry biosecurity survey

The survey response rate was deemed acceptable (50%, 25 of 50 surveys sent). One respondent returned the survey unanswered as they were a manufacturer of rabbit equipment only. The remaining 24 surveys were completed; however, in some cases respondents opted not to answer one or more individual questions. Frequently, multiple answers were provided for 1 question by a single respondent; these were all included in the results. Farm size ranged from 6 does to > 900 (mean = 168; median = 100; SD = 194). Farms had been in business for 1 to 40 y; the mean time in operation was 10 y and the median time was 8 y. Regardless of size, all facilities had between 1 to 5 employees, with many facilities having only 1 to 2 part-time employees. In all cases, rabbits were being raised for meat; however, occasionally they were also being sold for pet or research use, or as breeding stock. A total of 16 small farms and 8 large farms were identified. The average number of breeding does kept on small farms was 68 and on large farms was 368.

Responses to questions applicable to the 3 major areas of biosecurity (access management, animal health management, and operation management) follow. Significant differences based on farm size were only observed for 2 biosecurity/husbandry questions: "Do employees wash their hands before leaving the barn?" and "Are feeders and feed bowls cleaned and/or disinfected monthly?" The odds of employees washing their hands after leaving the barn were significantly lower for small farms than for large farms (OR = 0.104; 95% CI = 0.012 to 0.842; $P = 0.026$). The odds of feeders being cleaned monthly were significantly lower for small farms than for large farms (OR = 0.0604; 95% CI = 0.0019 to 0.632; $P = 0.017$); however, there was no significant difference between any other cleaning frequencies (weekly, yearly, etc.) so the clinical significance of this result is unknown. In an additional question, "Do you feed your animals hay?" there was a trend ($P = 0.054$) for small farms to be more likely to feed hay than large farms. No significant differences existed between small and large farms for any other questions in the survey and all other results were combined.

In general, few biosecurity measures were routinely implemented and few producers had a good level of awareness of biosecurity practices. In terms of access management, all respondents visited other farms (including other sites that they owned or at which they kept animals), with 29% (6/21) indicating that this occurred daily. All facilities reported few visitors; however, of the 71% (17/24) of facilities that had visitors, the visitors frequently came from other farms, including rabbitries. In most cases, there were no specific procedures in place for visitor entry or rabbit handling. Livestock species other than rabbits were kept on the same farm by 63% (15/24) of respondents, and 53% of these were kept in the same barn as the rabbits.

Despite having relatively small numbers of employees, not all employees were trained to recognize signs of disease in rabbits. Only 63% (15/24) of the facilities kept a mortality log for rabbits. No facility implemented all the barn procedure protocols (e.g., anteroom, handwashing, dedicated barn clothing and equipment), and the most frequently practiced protocol, restricted barn access, was practiced in only 63% (12/19) of facilities. Quarantine for new animals, which were brought into 46% (11/24) of farms, was performed in 55% (6/11) of farms.

In terms of operations management, the primary method of carcass disposal was composting (50%; 12/24). Most producers (75%; 18/24) indicated that they disposed of manure and litter via a manure pile, and 46% (11/24) spread this manure on fields. Seventy-one percent (17/24) of farms relied on negative pressure for barn ventilation. For does and bucks, cages were cleaned in 75% (18/24) and 67% (16/24) of barns, respectively, and for both groups, 50% of respondents indicated that cages were cleaned on an irregular basis. Fryer cages were also cleaned in 75% (18/24) of respondent farms, most frequently (44%) on a monthly basis.

Discussion

In 2015, the National Farm Animal Care Council (NFACC), announced its intention to develop a Code of Practice for rabbits to address welfare issues within the Canadian meat rabbit industry (22). This new code will provide rabbit producers with husbandry, management, and handling guidelines to enhance rabbit care in production settings. The results from the current study will allow NFACC to benchmark current industry practices and focus on highlighting methods to improve animal health and welfare by reducing infectious enteric disease and enhancing on-farm biosecurity practices.

In this study, causes of REC in Ontario meat rabbits could be attributed to infectious organisms in at least 65% of cases, based on microbiology and histopathology findings. Of these cases, 80% were caused by bacterial species that are common pathogens of humans and other animals, suggesting that rabbit to human and rabbit to other animal bacterial transmission is

Table 3. Results of 3-way simple effects analysis for differences between least squares mean estimates of intestinal histopathology scores (i.e., estimated marginal mean) in meat rabbits with variables A and B held constant and variable C compared in pairwise fashion

Variable A	Variable B	Variable C	Estimated marginal mean	Lower limit	Upper limit	*P*-value (*significant at $P \leq 0.05$)
Season (Spring)	Sample site (cecum)	Age (doe *versus* fryer)	−1.62	−2.68	−0.55	< 0.01*
	Sample site (colon)	Age (doe *versus* fryer)	−1.28	−2.36	−0.20	0.02*
	Sample site (ileum)	Age (doe *versus* fryer)	−1.51	−2.83	−0.19	0.02*
	Sample site (jejunum)	Age (doe *versus* fryer)	−1.97	−3.03	−0.91	< 0.01*
	Age (doe)	Sample site (cecum *versus* colon)	0.80	−0.09	1.70	0.08
		Sample site (cecum *versus* ileum)	1.65	0.57	2.73	< 0.01*
		Sample site (cecum *versus* jejunum)	1.40	0.53	2.27	< 0.01*
		Sample site (colon *versus* ileum)	0.85	−0.24	1.94	0.13
		Sample site (colon *versus* jejunum)	0.60	−0.30	1.49	0.19
		Sample site (ileum *versus* jejunum)	−0.25	−1.33	0.83	0.65
	Age (fryer)	Sample site (cecum *versus* colon)	1.14	0.41	1.88	< 0.01*
		Sample site (cecum *versus* ileum)	1.75	0.89	2.62	< 0.01*
		Sample site (cecum *versus* jejunum)	1.05	0.31	1.78	0.01*
		Sample site (colon *versus* ileum)	0.61	−0.25	1.47	0.16
		Sample site (colon *versus* jejunum)	−0.10	−0.83	0.64	0.80
		Sample site (ileum *versus* jejunum)	−0.71	−1.57	0.16	0.11
Season (Winter)	Sample site (cecum)	Age (doe *versus* fryer)	−1.52	−2.63	−0.40	0.01*
	Sample site (colon)	Age (doe *versus* fryer)	−1.43	−2.55	−0.32	0.01*
	Sample site (ileum)	Age (doe *versus* fryer)	−2.00	−3.18	−0.82	< 0.01*
	Sample site (jejunum)	Age (doe *versus* fryer)	−3.94	−5.08	−2.80	< 0.01*
	Age (doe)	Sample site (cecum *versus* colon)	0.92	−0.06	1.89	0.06
		Sample site (cecum *versus* ileum)	1.20	0.17	2.24	0.02*
		Sample site (cecum *versus* jejunum)	1.33	0.32	2.33	0.01*
		Sample site (colon *versus* ileum)	0.29	−0.75	1.32	0.58
		Sample site (colon *versus* jejunum)	0.41	−0.59	1.41	0.42
		Sample site (ileum *versus* jejunum)	0.12	−0.93	1.17	0.82
	Age (fryer)	Sample site (cecum *versus* colon)	1.00	0.38	1.62	< 0.01*
		Sample site (cecum *versus* ileum)	0.72	0.077	1.37	0.03*
		Sample site (cecum *versus* jejunum)	−1.10	−1.72	−0.48	< 0.01*
		Sample site (colon *versus* ileum)	−0.28	−0.92	0.37	0.40
		Sample site (colon *versus* jejunum)	−2.10	−2.72	−1.48	< 0.01*
		Sample site (ileum *versus* jejunum)	−1.82	−2.47	−1.18	< 0.01*
Age (doe)	Sample site (cecum)	Season (spring *versus* winter)	0.90	−0.62	2.41	0.24
	Sample site (colon)	Season (spring *versus* winter)	1.01	−0.52	2.54	0.19
	Sample site (ileum)	Season (spring *versus* winter)	0.45	−1.22	2.13	0.59
	Sample site (jejunum)	Season (spring *versus* winter)	0.82	−0.71	2.36	0.29
Age (fryer)	Sample site (cecum)	Season (spring *versus* winter)	0.61	−0.26	2.26	0.12
	Sample site (colon)	Season (spring *versus* winter)	0.61	−0.41	2.12	0.17
	Sample site (ileum)	Season (spring *versus* winter)	0.66	−1.38	1.31	0.96
	Sample site (jejunum)	Season (spring *versus* winter)	0.61	−2.41	0.11	0.07

possible (and *vice versa*) if biosecurity practices are poor. For example, *Lawsonia intracellularis* has been identified as a causative agent of several enteropathies in various species, including rabbits, pigs, hamsters, horses, ferrets, and canids (23). Transmission of *L. intracellularis* between rabbits and foals has been demonstrated experimentally (24); therefore, these species should be housed separately to avoid potential interspecies disease transmission. Similarly, *Salmonella* Agona, a *Salmonella enterica* serovar that was first isolated in cattle in 1950, was identified in 1 clinically healthy doe in this study. Transmission of this bacterium to rabbits has been demonstrated to occur either directly from infected animals or *via* contaminated feed (25). There are no reported cases of transmission of *Salmonella* spp. between rabbits and humans but reports exist for spread of this agent between humans and other species, such as cattle, pigs, and poultry, either directly or *via* contaminated meat or other food products, thus the potential for zoonotic spread of this agent cannot be ignored (26–28).

Differing patterns of bacterial enterocolitis were observed between the winter and spring cohorts and between the fryers and does, with significantly more cases of EPEC infection present during the winter months in fryers. While *eae*-positive *E. coli* can be found in low numbers in clinically normal rabbits, they have also been associated with enteritis and are considered a significant cause of morbidity and mortality (5,29–31). The presence of EPEC in rabbits in this study is especially concerning as EPEC may infect humans, causing enteritis. Close clonal relationships have been identified in EPEC isolated from numerous animals and humans and they are thought to be readily transmitted from humans to animals, and *vice versa* (32). While there are no known reports of direct transmission of EPEC from rabbits to humans, there has been at least 1 experimental study demonstrating transmission of human and rabbit EPEC strains to pigs (33,34). To more fully understand the biosafety risk to humans and other species imposed by the specific EPEC strains identified in rabbits in this study, further characterization of the

Table 4. Summary of rabbit cecal bacterial isolates

Bacterial species isolated	Spring 2007			Winter 2007/2008		
	Number isolated in fryers (%) $n = 15$	Number isolated in does (%) $n = 21$	Total number identified (%) $n = 36$	Number isolated in fryers (%) $n = 31$	Number isolated in does (%) $n = 12$	Total number identified (%) $n = 43$
Salmonella spp.	0 (0)	0 (0)	0 (0)	0 (0)	1 (8)[a]	1 (2.3)
C. perfringens	0 (0)	0 (0)	0 (0)	1 (3.2)	0 (0)	1 (2.3)
C. difficile	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
L. intracellularis	1 (6.7)	0 (0)	1 (2.8)	3 (10)	0 (0)	3 (7)
C. spiroforme	4 (26.7)[c]	0 (0)[c]	4 (11.1)	5 (16)[c]	1 (8)[c]	6 (14)
E. coli — all types	9 (60.0)	5 (23.8)	14 (38.9)	15 (48)	6 (50)	21 (49)
— *eae* (EPEC)	1 (6.7)[c]	0 (0)[c]	1 (2.8)[b]	9 (29)[c]	1 (8)[c]	10 (23)[b]
— *stx1*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
— *stx2*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
— VTEC	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[a] *Salmonella* Agona.
[b] Statistically significant difference between winter and spring, $P < 0.05$.
[c] Statistically significant difference between fryers and does when combined for season, $P < 0.05$.
stx1 — Shiga toxin type 1 gene; *stx2* — Shiga toxin type 2 gene; *eae* — gene for intimin.

strains is needed to identify their specific characteristics (35). No enterohemorrhagic *E. coli* (EHEC) strains were detected in any of the rabbits in this study.

The increased likelihood of fryers to be infected with EPEC and *C. spiroforme* is consistent with European studies, in which suckling and weanling rabbits were more susceptible to enteric diseases (29). Younger rabbits have a less developed immune system, a higher gastric pH, and are often fed relatively carbohydrate dense diets to maximize growth rates, all potentially contributing to disruptions in normal microbiota and dysbiosis with overgrowth of opportunistic pathogens. Given that rabbits are hind gut fermenters with a massive cecal bacterial burden, it is not surprising that the highest microscopic lesion scores largely occurred in this tissue in both fryers and does.

There are several potential causes for the increased prevalence of infectious agents and enteritis during the winter months, including difficulty in maintaining adequate barn ventilation while maintaining an appropriate ambient temperature. This often can result in poorer ventilation during cold, wet conditions as air inlets and outlets, windows, and doors (all identified as common means of ventilation in the survey) are more likely to be closed, and fans turned off during the winter months. Decreased ventilation, combined with irregular cage cleaning and disinfection, may result in increased ammonia levels, pulmonary injury, and overall declines in immunity (36,37).

The presence of potential zoonotic pathogens in Ontario meat rabbit farms is of concern when the results of the biosecurity survey are considered. The identified lack of specific biosecurity measures to prevent disease transmission, regardless of farm size, significantly increases the risk of disease transfer, not just between rabbits, but also between rabbits and humans. Many of the respondents had employees who worked at other farms on a regular basis, further potentiating the possibility for disease spread between species on different farms. The results of the survey clearly demonstrate a need to develop and instill biosecurity measures for the meat rabbit industry. This should include washing hands before handling healthy and sick animals, use of dedicated barn clothing and equipment, and controlling farm and barn access. Implementing these measures will help to reduce risk of disease transmission among rabbits and between rabbits and humans.

This study was limited by several factors, the first being voluntary enrolment, which can skew the study population. By targeting larger Ontario producers, and specifically those who are likely to provide stock to the smaller producers, we felt that we were able to adequately control for this potential bias. In addition, the survey response rate of 50% suggested a reasonable representation of the study population. Secondly, this study commenced following an outbreak of gastroenteritis in the industry. At the time of sampling, some farms had been dealing with the problem for several months, including attempting to institute treatments, and the rabbits submitted for postmortem evaluation may have been different from those initially affected. Enteropathogenic *E. coli* has frequently been identified as a cause of enteritis in rabbits in other studies outside of Canada, and therefore, while it may not have been the sole agent responsible for this outbreak, it likely played a significant role. The current study focused exclusively on bacterial causes of enteritis. It is well-established that viruses, most commonly rotavirus and coronavirus, are associated with REC, and frequently in a multifactorial fashion (4,5). Studies examining the prevalence of these viruses and their association with enteric disease are required to gain a better understanding of REC in Canadian meat rabbits.

In conclusion, we identified some common causes of REC in Ontario meat rabbit farms, consisting largely of *E. coli, C. spiroforme,* and *L. intracellularis.* Biosecurity practices on rabbitries were uniformly poor, indicating a critical need for development and implementation of industry-wide biosecurity standards. As infectious disease was identified as a predominant cause of enteritis, the implementation of such measures will likely aid in decreasing morbidity and mortality in affected animals, and improving overall animal and human health and welfare, and farm productivity.

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